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Rapid Detection and Serotyping of Adenovirus by Direct Immunofluorescence

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Four fluorescent antibody reagents were evaluated for their suitability for the identification of adenovirus isolates by immunofluorescence. The antibodies used in the reagents consist of monoclonal antibodies against adenovirus type 3 (Ad3), Ad4, Ad8, and adenoviruses of subgroup C (Ad1,2,5,6), serotypes known to occur in outbreaks of disease. Most of the monoclonal antibodies employed were reactive against type-specific antigens found on the hexon protein. Reagents employing two noncompeting anti-hexon antibodies were more sensitive than reagents prepared with only one monoclonal antibody, although both types of reagents exhibited a high degree of specificity. Five hundred and seventeen adenovirus isolates (359 of which had previously been typed by other methods) and 46 nonadenovirus isolates were examined with all four type-specific reagents in parallel with an adenovirus group-specific reagent. The results indicate that direct typing of adenovirus isolates is feasible, leading to significant savings in time compared to other typing methods and should contribute to the management of certain adenovirus infections, particularly during outbreaks. ***J. Med. Virol. 51:198–201, 1997.***

KEY WORDS: adenovirus; monoclonal antibody; immunofluorescence

INTRODUCTION

At least 47 documented serotypes of adenoviruses have been isolated [Hierholzer et al., 1988] from patients in association with clinical conditions as diverse as respiratory, ocular, and gastrointestinal tract infection. The true contribution of adenoviruses to illness is probably double that estimated on the basis of virus isolation alone [Fox and Hall, 1980]. Traditionally, diagnosis of adenoviruses has been by virus isolation in cell culture, which can be time consuming [Lennette and Schmidt, 1979; Landry and Hsiung, 1986]. A number of procedures including haemagglutination inhibition (HAI), serum neutralisation (SN) employing hyperimmune type-specific animal antisera, and restriction endonuclease analysis (REA) of viral DNA are available for the identification of adenovirus isolates [Kasel, 1992]. SN is the system of choice for the identification of low-numbered and a limited number of higher-numbered serotypes and is commonly used in routine laboratories [Lipson et al., 1993]. HAI and REA are time consuming and not readily applicable to the routine laboratory. Therefore, rapid diagnostic techniques are needed to reduce the time taken by many laboratories to type isolates in order that results may be clinically useful [Gardner, 1977]. A popular rapid diagnostic method is the immunofluorescence assay (IFA) because it is simple to perform and can detect antigen in cell culture specimens as early as 2 days after inoculation, often before a cytopathic effect is visible. Additionally, IFA sensitivity and specificity can be equal to that of enzyme immunoassays (EIA) and REA [Nerurkar et al., 1987]. Currently group-specific monoclonal-based fluorescent reagents are used to detect adenoviruses, but type-specific reagents are not available routinely.

The detection and typing of particular adenovirus serotypes which occur in outbreaks can be of significant epidemiological importance. Adenovirus serotypes of interest include adenovirus type 8 (Ad8), a potential source of hospital-based outbreaks of epidemic keratoconjunctivitis [Barnard et al., 1973; Tullo and Higgins, 1979]. Ad4 is another serotype capable of causing outbreaks of upper respiratory tract infection (acute respi-

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ratory disease) in military recruits [Hilleman and Werner, 1954] or conjunctivitis [D'Angelo et al., 1979; Tullo and Higgins, 1980; Levandowski and Rubenis, 1981]. Common endemic types such as adenovirus types 1, 2, 5, 6 are known to cause upper respiratory tract and eye infections in children exposed early in life and more importantly, these serotypes can persist for long periods [Fox et al., 1977; Gigliotti et al., 1981]. Ad3 has been largely identified with pharyngoconjunctival fever (PCF), a respiratory disease with mild ocular involvement which is highly infectious and known to cause outbreaks in schools and swimming baths [Ormsby and Aitchison, 1955; Martone et al., 1980]. Other serotypes have been reported in a few cases to cause PCF. Ad3 and Ad7 cause pneumonia in young children which can be life-threatening and require rapid diagnosis to allow appropriate control of infection procedures to be instigated. In order to diagnose such infections quickly, suitable reagents need to be made available for use in routine diagnostic laboratories.

In this investigation, four monoclonal IFA reagents reactive against adenoviruses in subgroup C (Ad1,2,5,6) Ad3, Ad4, and Ad8 [Russell et al., 1981; Sharp, 1988; Wood et al., 1994] were produced and evaluated for their ability to detect and type human adenovirus isolates from clinical material. A group-specific reagent described previously [Sharp and Bailey, 1988] was used in parallel with the typing reagents.

MATERIALS AND METHODS

Reagents

Monoclonal antibodies (MAbs) designated as 10/19.2 and 10/85.6 reactive against Ad3; MAb 7/48.7 reactive against Ad1, Ad2, Ad5, and Ad6; MAb 13.2.3 reactive against Ad4; MAbs 44.6 and 2E46.1.4 reactive against Ad8 were produced in the Laboratory of Microbiological Reagents (Central Public Health Laboratory, London). These all bind to the adenovirus hexon protein with the exception of MAb 13.2.3 which binds to the penton base protein [Sharp, 1988]. They were purified from ascitic fluids by protein A Sepharose CL-4B affinity chromatography, according to the method described by Ey et al. [1978]. Conjugation of the MAbs to fluorescein isothiocynate (FITC) was modified from the method described by Goding [1976] and Johnstone and Thorpe [1982]. For use, the conjugates were diluted to working strength in phosphate-buffered saline (PBS) containing 0.005% of Evans blue counterstain (BDH). Working strength was chosen as the highest dilution which gave maximum fluorescence.

Clinical Material

Five hundred and sixty-three isolates consisting of 359 adenoviruses typed previously, 46 nonadenoviruses, and 158 adenovirus isolates which had not been serotyped were tested. These isolates were obtained from different sources (305 eye swabs, 38 throat, 65 stool, 69 nasopharyngeal aspirates, 6 lung biopsies, 3 vesicle fluids, and 77 unrecorded specimens) in patients exhibiting varying clinical symptoms such as conjunctivitis, pyrexia, respiratory tract infection, diarrhoea, and other minor symptoms.

Virus Isolation

Each evaluating laboratory prepared specimens following its own standard procedure. In summary, HEp2, Graham 293, or MRC5 cell cultures were inoculated and then examined 48 hours later. Infected cells showing cytopathic effect were harvested and a cell suspension air dried onto Teflon-coated microscope slides, fixed in cold acetone, and stained with the different reagents. A 10 μl volume of reagent per well was used to ensure that the whole well was covered. The stained slide was incubated in a moist chamber at 37°C for 30 minutes. The slide was then washed with PBS twice for 10 minutes in total, briefly rinsed with deionised water, and partly air dried prior to examination. Prepared slides were then viewed immediately after staining.

Analysis of the Field Trial Data

The sensitivity, specificity, and the predictive values of the different IFA reagents were calculated by standard statistical formulae for analysing diagnostic tests [Altman, 1991]. For the analysis of specificity, SN and REA results of the isolates previously typed were used for comparison.

RESULTS

A summary of the data obtained in the evaluation is shown in Table I. All the isolates in the study were tested by the Ad group reagent and were all correctly identified as adenoviruses or nonadenoviruses. With the reagents used in this study, the Ad3 type-specific reagent had a sensitivity and specificity of 100% (137/137 and 268/268, respectively). The Ad8 type-specific reagent had a sensitivity and specificity of 100% (21/21 and 384/384, respectively), however, this latter result is not statistically significant because of the low number of Ad8 isolates. The Ad subgroup C reagent had a sensitivity of 84% (67/80) and a specificity of 100% (325/325). The Ad4 type-specific reagent had a sensitivity of 70% (33/47) and a specificity of 97% (346/358). The Ad4 reagent showed a ±fluorescence (faint but distinguishable from the background) with a few Ad3 ($n = 12$) isolates, but because the isolates were strongly positive with the Ad3 reagent, these were not wrongly identified.

The four type-specific reagents and the Ad group reagent did not cross-react with any of the nonadenovirus isolates tested ($n = 46$) such as respiratory syncytial virus, herpes simplex virus 1 and 2, varicella zoster virus, rhinovirus, cytomegalovirus, and chlamydia isolates or with the different cell lines used to grow these viruses. Examination of the 158 untyped isolates using the type-specific reagents resulted in 84 (53%) being typed successfully. Sixteen were typed as members of subgroup C (10%), 59 as Ad3 (37%), 3 as Ad4 (2%), and 6 as Ad8 (4%) with 74 isolates (45%) untyped.

TABLE I. Summary of the Evaluation of the Four Type-Specific Reagents

IFA reagent	No. of specimens detected[a]				Percentage[b]			
	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
Ad 1,2,5,6	67	325	0	13	84	100	100	96
Ad 3	137	268	0	0	100	100	100	100
Ad 4	33	358	0	14	73	100	100	96
Ad 8	21	384	0	0	100	100	100	100
Ad Group	359	46	0	0	100	100	100	100

[a]A total of 359 confirmed isolates and 46 nonadenovirus isolates were examined. TP, test positive; TN, test negative; FP, false positive; FN, false negative.
[b]Sensitivity = number of test-positive specimens/(number of test-positive specimens + number of false negative specimens) × 100. Specificity = number of test-negative specimens/(number of test-negative specimens + number of false positive specimens) × 100. PPV = positive predictive value. Proportion of patients with a positive test result who were correctly diagnosed. NPV = negative predictive value. Proportion of patients with a negative test result who were correctly diagnosed.

DISCUSSION

MAbs employed in diagnostic reagents for the detection of viral antigen should ideally be targeted against epitopes which are highly conserved, highly immunogenic, and well represented in the antigen pool. The hexon protein of adenoviruses meets these criteria. It is a major component of the adenovirus capsid which accounts for 46% of the total mass of the virion, is highly immunogenic, and expresses several type and group-specific antigenic determinants [Wilcox and Ginsberg, 1963; Pettersson, 1971].

With the appropriate anti-hexon antibodies, an increase in the sensitivity of an IFA reagent to detect specific epitopes can be enhanced using a pool of two or more noncompeting antibodies. This decreases the theoretical risk of failing to detect any strains which do not possess a specific epitope and also increases the number of epitopes which are fluorescent labelled. A net increase in the intensity of the signal of the reagent can be observed. Both the Ad3 and Ad8 reagents were pools of two noncompeting MAbs and these proved to be the most sensitive reagents in the panel. Such MAbs are regarded as ideal reagents for typing strains [Sharp and Wadell, 1994]. The subgroup C (Ad1,2,5,6) and Ad4-specific reagents were composed of single antibodies and found to be less sensitive with clinical isolates. The Ad4 MAb produced the least sensitive reagent, probably because the antibody is reactive against the penton base protein of the viral capsid which is less immunogenic than the hexon and constitutes a small portion of the viral capsid.

Our data show that Ad3 isolates were the most commonly detected serotype found in the eye, confirming previous findings [Sharp and Wadell, 1994]. With the 158 untyped adenovirus isolates examined, 57% were typed successfully as Ad1-6 or Ad8. The Public Health Laboratory Service Communicable Disease Surveillance Centre [COSC, 1993] reported that 59% of adenoviruses typed by English and Welsh laboratories between 1990 and 1993 were Ad1–6 or Ad8. This percentage was confirmed in our study.

In this study, only a limited range of adenovirus-typing reagents was available. There is a need to broaden the panel of reagents to include other prevalent serotypes, for example, Ad7,10,12,19,31,35, and 37, before typing by IFA can completely replace SN in routine settings. There will always be a need for SN and REA to type the less commonly isolated serotypes, and REA specifically for the detection of previously unknown serotypes or genomic variants. However, this study shows that direct identification of serotypes by IFA is a feasible alternative to typing methods currently used in routine laboratories, producing more rapid results which should benefit patient management.

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REFERENCES

Altman DG (1991): Diagnostic tests—determination of the sensitivity and specificity. In "Practical Statistics for Medical Research." London: Chapman and Hall, pp 409–411.

Barnard DL, Dean Hart JC, Marmion VJ, Clarke SKR (1973): Outbreak in Bristol of conjunctivitis caused by adenovirus type 8 and its epidemiology and control. British Medical Journal 2:165–169.

CDSC (1993): Respiratory Diseases Section, Personal Communication.

D'Angelo LJ, Hierholzer JC, Keenlyside RA, Anderson LJ, Martone WJ (1979): Pharyngoconjunctival fever caused by adenovirus type 4: Report of a swimming pool-related outbreak with recovery of virus from pool water. Journal of Infectious Diseases 140:42–47.

Ey PL, Prowse SJ, Jenkin CR (1978): Isolation of pure IgG_1, IgG_{2a} and IgG_{2b} Immunoglobulins from mouse serum using Protein-A Sepharose. Immunochemistry 15:429–436.

Fox JP, Hall CE (1980): "Viruses in Families." Littleton, MD: PSG.

Fox JP, Hall CE, Coony MK (1977): The Seattle Virus Watch VII. Observations of adenovirus infections. American Journal of Epidemiology 105:362–386.

Gardner PS (1977): Rapid virus diagnosis. Journal of General Virology 36:1–29.

Gigliotti F, Williams WT, Hayden FG, Hendley JO, Benjamin J, Dickens M, Gleason C, Perriello VC, Wood J (1981): Etiology of acute conjunctivitis in children. Journal of Pediatrics 98:531–536.

Goding JW (1976): Conjugation of antibodies with fluorochromes: Modification to the standard methods. Journal of Immunological Methods 13:215–226.

Hierholzer JC, Wigand R, Anderson LJ, Adrain T, Gold JWM (1988): Adenovirus from patients with AIDS: A plethora of serotypes and a description of five new serotypes of subgroup D (types 43–47). Journal of Infectious Diseases 158:804–813.

Hilleman MR, Werner JR (1954): Recovery of a new agent from patients with acute respiratory illness. Proceedings of the Society for Experimental Biology and Medicine 85:183–188.

Johnstone A, Thorpe R (1982): Conjugation of fluorochromes to immunoglobulins. In "Immunochemistry in Practice." Oxford: Blackwell Scientific, pp 258–259.

Kasel JA (1992): Adenoviruses. In Lennette EH (ed): "Laboratory Diagnosis of Viral Infections." New York: Marcel Dekker, pp 223–242.

Landry ML, Hsiung GD (1986): Primary isolation of viruses. In Specter S, Lancz GJ (eds): "Clinical Virology Manual." Amsterdam: Elsevier, p 31.

Lennette EH, Schmidt NJ (1979): "Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections," 5th ed. Washington, DC: American Public Health Associates Inc., pp 229–255.

Levandowski RA, Rubenis M (1981): Nosocomial conjunctivitis caused by adenovirus type 4. Journal of Infectious Diseases 143:28–31.

Lipson SM, Iqbal AP, Ashley RL, Grady LJ, Ciamician Z, Teichberg S (1993): Presumptive identification of common adenovirus serotypes by the development of differential cytopathic effects in the human lung carcinoma (A549) cell culture. FEMS Microbiology Letters 113:175–182.

Martone WJ, Hierholzer JC, Keenlyside RA, Fraser DW, D'Angelo LJ, Winker WG (1980): An outbreak of adenovirus type 3 disease at a private recreation centre swimming pool. American Journal of Epidemiology 111:229–237.

Nerurkar LS, Miller NR, Namba M, Brashears G, Scherba G, Sever JL (1987): Typing of herpes simplex virus by capture biotin-streptavidin enzyme-linked immunosorbent assay and comparison with restriction endonuclease analysis and immunofluorescence method using monoclonal antibodies. Journal of Clinical Microbiology 25:128–132.

Ormsby HL, Aitchison WS (1955): The rule of the swimming pool in the transmission of pharyngo-conjunctual fever. J. Can. Med. Assoc. 73:864–866.

Pettersson U (1971): Structural proteins of adenoviruses. VI. On the antigenic determinants of the hexon. Virology 43:123–136.

Russell WC, Patel G, Precious B, Sharp I, Gardner PS (1981): Monoclonal antibodies against adenovirus type 5: Preparation and preliminary characterization. Journal of General Virology 56:393–408.

Sharp IR (1988): Production of Monoclonal Antibodies to Adenovirus with a view of their use as a diagnostic reagent, PhD Thesis, University of London.

Sharp IR, Bailey A (1988): Development of a diagnostic reagent for the detection of adenovirus antigen by immunofluorescence. Analytical Proceedings 25:129–131.

Sharp I, Wadell G (1994): Adenoviruses. In Zuckerman AJ, Banatvala JE, Pattison JR (eds): "Principles and Practice of Clinical Virology," 3rd ed. New York: John Wiley and Sons, pp 267–287.

Tullo AB, Higgins PG (1979): An outbreak of adenovirus keratoconjunctivitis in Bristol. British Journal of Ophthalmology 63:621–626.

Tullo AB, Higgins PG (1980): An outbreak of adenovirus type 4 conjunctivitis. British Journal of Ophthalmology 64:489–493.

Wilcox WC, Ginsberg HS (1963): Structure of type 5 adenovirus. I. Antigenic relationship of virus structural proteins to virus specific soluble antigens from infected cells. Journal of Experimental Medicine 118:295–306.

Wood SR, Sharp IR, deJong JC, Verweij-Uijterwaal MW (1994): The development and preliminary evaluation of an enzyme immunosorbent assay for the detection of adenovirus type 8. Journal of Medical Virology 44:348–352.